

## RESEARCH LETTERS

GII.6, GI.3/GII.17, GI.7/GII.17, and GI.7/GII.P7-GII.6). We did not perform dual typing on GI strains because they were less predominant than GII strains. However, future analysis of their recombination patterns will be useful for better characterizing these rare but potentially significant genotypes. This study was somewhat limited by lack of detailed clinical information accompanying the submitted samples and absence of surveillance from southern Thailand ( $\approx 14\%$  of the country's population). Molecular epidemiology and continued surveillance of norovirus strain diversity will increase awareness among clinicians and help epidemiologists determine global transmission patterns.

This work was supported by the Research Chair Grant from the National Science and Technology Development Agency (P-15-50004) and the Center of Excellence in Clinical Virology of Chulalongkorn University and Hospital (GCE 59-009-30-005). Support for W.C. was provided by the Tuition/Fee and Stipend Scholarship for Graduate Students (Faculty of Medicine of Chulalongkorn University) and a scholarship from Chulalongkorn University Graduate School to commemorate the Celebrations on the Auspicious Occasion of Her Royal Highness Princess Maha Chakri Sirindhorn's 60th Birthday.

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### References

- Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:725–30. [http://dx.doi.org/10.1016/S1473-3099\(14\)70767-4](http://dx.doi.org/10.1016/S1473-3099(14)70767-4)
- Vinje J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol*. 2015;53:373–81. <http://dx.doi.org/10.1128/JCM.01535-14>
- Chan MCW, Hu Y, Chen H, Podkolzin AT, Zaytseva EV, Komano J, et al. Global spread of norovirus GII.17 Kawasaki 308, 2014–2016. *Emerg Infect Dis*. 2017;23:1359–1354. <http://dx.doi.org/10.3201/eid2308.161138>
- Niendorf S, Jacobsen S, Faber M, Eis-Hübingen AM, Hofmann J, Zimmermann O, et al. Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016. *Euro Surveill*. 2017;22:30447. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30447>
- van Beek J, de Graaf M, Al-Hello H, Allen DJ, Ambert-Balay K, Botteldoorn N, et al.; NoroNet. Molecular surveillance of norovirus, 2005–16: an epidemiological analysis of data collected from the NoroNet network. *Lancet Infect Dis*. 2018;18:545–53. [http://dx.doi.org/10.1016/S1473-3099\(18\)30059-8](http://dx.doi.org/10.1016/S1473-3099(18)30059-8)
- Thanusuwannasak T, Puenpa J, Chuchaona W, Vongpunsawad S, Poovorawan Y. Emergence of multiple norovirus strains in Thailand, 2015–2017. *Infect Genet Evol*. 2018;61:108–12. <http://dx.doi.org/10.1016/j.meegid.2018.03.021>
- Debbink K, Costantini V, Swanstrom J, Agnihothram S, Vinje J, Baric R, et al. Human norovirus detection and production, quantification, and storage of virus-like particles. *Curr Protoc Microbiol*. 2013;31:15K.1.1–15K.1.45.
- Yang Z, Vinje J, Kulka M. Complete genome sequence of human norovirus GII.Pe-GII.4 Sydney from the United States. *Genome Announc*. 2017;5:e00159–17. <http://dx.doi.org/10.1128/genomeA.00159-17>
- Botha JC, Taylor MB, Mans J. Comparative analysis of South African norovirus GII.4 strains identifies minor recombinant variants. *Infect Genet Evol*. 2017;47:26–34. <http://dx.doi.org/10.1016/j.meegid.2016.11.004>

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## *Sneathia amnii* and Maternal Chorioamnionitis and Stillbirth, Mozambique

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DOI: <https://doi.org/10.3201/eid2508.190526>

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We report a case of *Sneathia amnii* as the causative agent of maternal chorioamnionitis and congenital pneumonia resulting in a late fetal death in Mozambique, with strong supportive postmortem molecular and histopathologic confirmation. This rare, fastidious gram-negative coccobacillus has been reported to infrequently cause abortions, stillbirths, and neonatal infections.

*Sneathia amnii*, formerly designated *Leptotrichia amnionii*, is a rare, fastidious, gram-negative coccobacillus, first described in the amniotic fluid of a woman with a fetal demise (1). The inherent difficulties in conventionally culturing this pathogen led to its initial identification through analyzing the 16S rRNA gene; its genome was recently sequenced (1,2). *S. amnii* is an opportunistic agent of the female urogenital tract (3,4) associated with cases of spontaneous abortion (miscarriage) and neonatal meningitis (1,5,6). We describe a perinatal case of *S. amnii* infection in a mother–fetus dyad, which we documented and investigated with the minimally invasive tissue sampling (MITS) postmortem procedure (7).

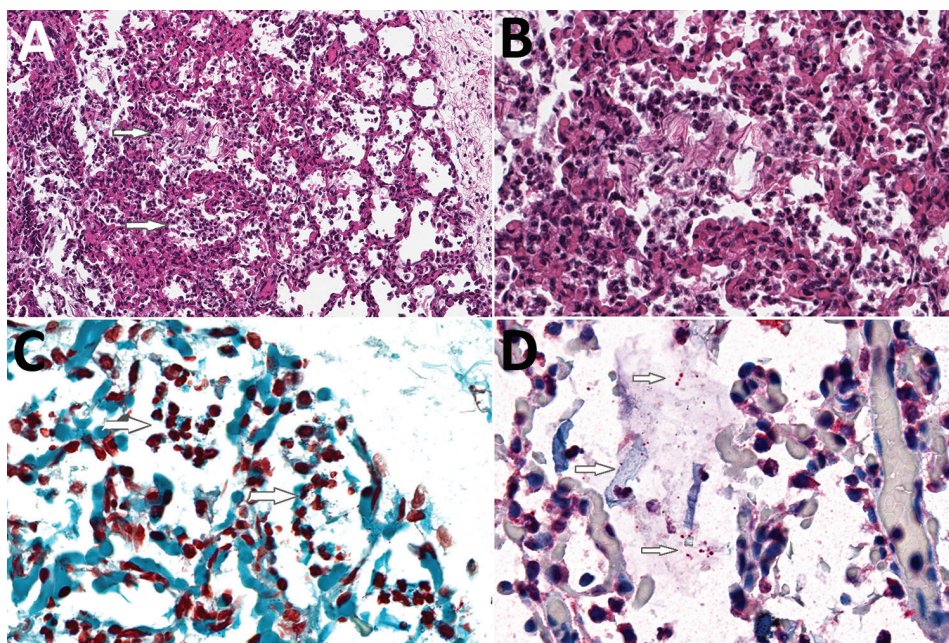
An otherwise healthy multigravida 37-year-old woman, at an estimated gestational age of 39 weeks, was admitted to Manhica District Hospital, southern Mozambique, in labor. During pregnancy, she had attended 2 antenatal consultations and received the standard of care for pregnant women in Mozambique; mild anemia was treated with ferrous sulfate and folic acid supplements. Serologic tests for syphilis and HIV were both negative. Upon arrival at the hospital, the mother was afebrile and hemodynamically stable; she had a fully effaced uterine cervix, thin and elastic, 2 cm dilation;

intact amniotic membranes; and cephalic fetal presentation with heartbeat present. Physical examination did not provide additional information. Labor progressed with spontaneous rupture of membranes. No additional documentation of the fetal heartbeat was available before delivery. Two hours after arrival, a fresh stillborn female weighing 3.5 kg was born by spontaneous vaginal delivery. Size was normal, and no macroscopic congenital abnormalities were observed. The mother was discharged next day without complications.

As part of Mozambique's Child Health and Mortality Prevention Surveillance (CHAMPS), after obtaining written, informed consent, we conducted MITS by biopsy needle of tissues and body fluids, in addition to placenta, to ascertain the cause of the stillbirth (7). Samples are subject to thorough histopathologic, molecular, and microbiological investigation, including universal screening for HIV-1, *Mycobacterium tuberculosis*, and malaria parasites. We performed conventional microbiological cultures of blood and cerebrospinal fluid (CSF); we inoculated  $\approx 3$  mL of blood into aerobic blood culture bottle (BACTEC system; Becton Dickinson, <https://www.bd.com>) and cultured CSF samples into blood, chocolate, and MacConkey agar plates. We performed multipathogen molecular screening using TaqMan Array Card (Applied Biosystems, <https://www.thermofisher.com>) in whole blood, CSF, lung, and rectal swab samples (8). We prepared and examined tissue samples using conventional pathologic methods and targeted immunohistochemical staining (9).

We isolated no microorganisms in CSF or blood, nor did we detect a likely pathogen in any of the unfixed

**Figure.** Histologic evidence of amniotic fluid aspiration, bronchopneumonia, and intraalveolar gram-negative coccobacilli in the lung of a stillborn infant, Mozambique. A) Hematoxylin and eosin stain of lung tissue showing acute inflammation within alveoli (bronchopneumonia, upper arrow) and moderate numbers of aspirated squames (lower arrow), consistent with intrauterine fetal distress and associated aspiration of amniotic fluid. Original magnification  $\times 20$ . B) Higher magnification of panel A tissue showing acute inflammation within alveoli (bronchopneumonia) and a clump of aspirated squames. Original magnification  $\times 40$ . C) Gram stain of lung showing multiple small, gram-negative coccobacilli mixed with acute inflammation within alveoli (arrows indicate regions with bacteria). Original magnification  $\times 63$ . D) Polybacterial immunohistochemical assay of lung tissue targeting multiple bacteria highlights the coccobacilli within alveoli (top and bottom arrows). Aspirated squames are also present (middle arrow). Original magnification  $\times 63$ .





postmortem tissues. At CHAMPS reference pathology laboratories, examination of tissue samples showed similar morphological findings in placental and miscellaneous tissues that suggested infection, including an acute inflammatory infiltrate in the lungs compatible with bronchopneumonia. We also found moderate numbers of aspirated squames and increased alveolar macrophages, indicating intrauterine fetal distress and associated aspiration of amniotic fluid. No aspirated meconium was apparent. Gram stain revealed gram-negative coccobacilli in alveoli and adjacent bronchioles. We conducted a cross-reactive immunohistochemical assay targeting multiple bacteria in the lung samples using paneubacteria and gram type-specific PCR assays targeting the 16S rRNA gene; we identified *S. amnii* by sequence analysis of positive amplicons (Figure, panels A–C). We observed no remarkable histopathologic findings in the liver or brain, and the cross-reactive polybacterial immunohistochemical assay was negative in brain tissue. Placental tissue and umbilical cord showed an acute chorioamnionitis with maternal response (inflammation in the membranes, stage 2) and fetal response (inflammation in the umbilical cord, stage 2) showing umbilical arteritis with rare gram-negative coccobacilli. There was no immunohistochemical evidence of bacteria in this tissue (Figure 1, panel D). We obtained an amplicon from placental tissue by paneubacteria PCR; however, we could not confirm the presence of *S. amnii* sequences.

CHAMPS procedures include the review of all clinical, microbiological, molecular, and histopathological data, along with the verbal autopsy, by a multidisciplinary panel of local experts (D.M. Blau et al., unpub. data). The panel concluded that the immediate cause of this stillbirth could be attributed to a congenital pneumonia, caused by *S. amnii*, that could have originated in the mother's placenta; we determined that chorioamnionitis was the main maternal condition associated with the child's death. The presence of *Sneathia* sp. bacteria in amniotic fluid can lead to inflammation and histologic chorioamnionitis, amnionitis, or both (10).

*S. amnii* has been identified in different settings as a pathologic agent in women and children (1,3–6). In this case in a rural setting in Africa, *S. amnii* was the causative agent in a stillbirth with congenital pneumonia, a diagnosis supported by strong postmortem molecular and histopathologic confirmation. As CHAMPS evaluation continues in Mozambique, as well as at sites in 6 additional countries in sub-Saharan Africa and south Asia, we expect the importance of this pathogen to become clearer.

The Spanish Agency of Cooperation and International Development (AECID) funds the core activities of Centro de Investigação em Saúde de Manhiça. R.V. had a fellowship from the Rio Hortega program of the Instituto de Salud Carlos III (ISCIII) grant no. CD16/00024.

J.M.G. received grants from the Gates Foundation during the study period. The other authors declare that they have no competing interests.

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## References

- Shukla SK, Meier PR, Mitchell PD, Frank DN, Reed KD. *Leptotrichia amnionii* sp. nov., a novel bacterium isolated from the amniotic fluid of a woman after intrauterine fetal demise. *J Clin Microbiol*. 2002;40:3346–9. <https://doi.org/10.1128/JCM.40.9.3346-3349.2002>
- Harwich MD Jr, Serrano MG, Fettweis JM, Alves JM, Reimers MA, Buck GA, et al.; Vaginal Microbiome Consortium (additional members). Genomic sequence analysis and characterization of *Sneathia amnii* sp. nov. *BMC Genomics*. 2012;13(Suppl 8):S4. <https://doi.org/10.1186/1471-2164-13-S8-S4>
- Gundi VA, Desbriere R, La Scola B. *Leptotrichia amnionii* and the female reproductive tract. *Emerg Infect Dis*. 2004;10:2056–7. <https://doi.org/10.3201/eid1011.031019>
- Thielsen CM, Nicolaidis M, Lökebö JE, Falsen E, Jorde AT, Müller F. *Leptotrichia amnionii*, an emerging pathogen of the female urogenital tract. *J Clin Microbiol*. 2007;45:2344–7. <https://doi.org/10.1128/JCM.00167-07>
- Boennelycke M, Christensen J, Arpi M, Krause S. *Leptotrichia amnionii* found in septic abortion in Denmark. *Scand J Infect Dis*. 2007;39:382–3. <https://doi.org/10.1080/00365540601053022>
- Decroix V, Goudjil S, Kongolo G, Mammeri H. '*Leptotrichia amnionii*', a newly reported cause of early onset neonatal meningitis. *J Med Microbiol*. 2013;62:785–8. <https://doi.org/10.1099/jmm.0.051870-0>
- Menendez C, Castillo P, Martínez MJ, Jordao D, Lovane L, Ismail MR, et al. Validity of a minimally invasive autopsy for cause of death determination in stillborn babies and neonates in Mozambique: an observational study. *PLoS Med*. 2017;14:e1002318. <https://doi.org/10.1371/journal.pmed.1002318>
- Diaz MH, Waller JL, Theodore MJ, Patel N, Wolff BJ, Benitez AJ, et al. Development and implementation of multiplex TaqMan array cards for specimen testing at Child Health and Mortality Prevention Surveillance (CHAMPS) site laboratories. *Clin Infect Dis*. In press 2019.
- Martines RB, Ritter JM, Gary J, Shieh W-J, Ordi J, Hale M, et al. Pathology and telepathology methods in the Child Health and Mortality Prevention Surveillance Network (CHAMPS). *Clin Infect Dis*. In press 2019.
- Han YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. *J Clin Microbiol*. 2009;47:38–47. <https://doi.org/10.1128/JCM.01206-08>

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